

AWARD NUMBER:

W81XWH-15-1-0598

TITLE:

Development of Small Molecule Activators of Protein Phosphatase 2A (SMAPs)
for the Treatment of Castration-Resistant Prostate Cancer

PRINCIPAL INVESTIGATOR:

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REPORT DATE:

October 2016

TYPE OF REPORT:

Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE October 2016		2. REPORT TYPE Annual		3. DATES COVERED 30 Sep 2015 - 29 Sep 2016	
4. TITLE AND SUBTITLE Development of Small Molecule Activators of Protein Phosphatase 2A (SMAPs) for the Treatment of Castration-Resistant Prostate Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-15-1-0598	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Stephen Plymate E-Mail: splymate@uw.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Washington Seattle, WA 98104-2499				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Subject: Protein phosphatase 2A (PP2A) is among the most abundant serine threonine phosphatases in mammalian cells, a bona fide tumor suppressor, and a key negative regulator of critical oncogenic proteins including the androgen receptor (AR), Akt, Erk, and Myc. We have recently developed a series of small molecules that activate PP2A and thereby exert anticancer effects in cell culture and xenograft models. This proposal focuses on a third generation, orally bioavailable small molecule activator of PP2A (SMAP), DT-061, with improved potency and pharmacologic properties compared to our earlier series. Activation of PP2A represents a highly novel approach to cancer treatment that may coordinately downregulate the AR and other key PP2A regulated oncogenic pathways. Purpose: We hypothesize that our novel derivative DT-061 activates PP2A, downregulates key PP2A substrates, and confers anti-prostate cancer activity. The objectives of this proposal are to further probe the mechanism and activity of DT-061 in anticipation of advancing this novel approach to cancer treatment into the clinic. Scope of Research: Determine the effects of DT-061 on clinically relevant patient-derived xenograft models of prostate cancer, representing various disease states and resistance mechanisms. These models will be utilized to determine the effects of DT-061 on tumor growth and the pharmacodynamic effects of treatment.					
15. SUBJECT TERMS PP2A, androgen receptor, prostate cancer, small molecule, patient derived xenograft					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	15	19b. TELEPHONE NUMBER (include area code)

TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	4
2. Keywords	4
3. Accomplishments	5
4. Impact	7
5. Changes/Problems	8
6. Products	10
7. Participants & Other Collaborating Organizations	12
8. Special Reporting Requirements	15
9. Appendices	15

1. INTRODUCTION:

Subject: Protein phosphatase 2A (PP2A) is among the most abundant serine threonine phosphatases in mammalian cells, a bona fide tumor suppressor, and a key negative regulator of critical oncogenic proteins including the androgen receptor (AR), Akt, Erk, and Myc. Indeed, decreased PP2A activity and/or reduced expression of PP2A enzyme subunits has previously been demonstrated in multiple malignancies including prostate cancer. We have recently developed a series of small molecules that activate PP2A and thereby exert anticancer effects in cell culture and xenograft models. This proposal focuses on a third generation orally bioavailable small molecule activator of PP2A (SMAP), DT-061, with improved potency and pharmacologic properties compared to our earlier series. We have demonstrated, as detailed in the application, that our SMAPS induce apoptosis in prostate cancer cell lines and dephosphorylate and degrade the AR. Activation of PP2A represents a highly novel approach to cancer treatment that may coordinately downregulate the AR and other key PP2A regulated oncogenic pathways. This project represents a multi-disciplinary collaboration - the principal investigators have been working together on the development of these novel compounds for 3 years and bring the complementary expertise and experience necessary for successful completion of this project. **Purpose:** We hypothesize that our novel derivative DT-061 activates PP2A, downregulates key PP2A substrates, and confers anti-prostate cancer activity. The objectives of this proposal are to further probe the mechanism and activity of DT-061 in anticipation of advancing this novel approach to cancer treatment into the clinic. **Scope of Research:** **Aim 1:** Determine the effects of DT-061 on clinically relevant patient-derived xenograft models of prostate cancer, representing various disease states and resistance mechanisms (e.g., castration-sensitive and resistant, enzalutamide-sensitive and resistant, etc). These models will be utilized to determine the effects of DT-061 on tumor growth and the pharmacodynamic effects of treatment. **Aim 2:** Determine the effects of DT-061 on the phosphoproteome in vivo in tumors treated in Aim 1. Global phosphoproteomic profiling will be performed to define the critical phosphoproteomic perturbations. **Aim 3:** Probe the effects of DT-061 on the AR and other PP2A substrates in prostate cancer cells and demonstrate the effects occur in a PP2A-dependent manner. We will determine the impact of PP2A inhibition and proteasome inhibition on SMAP-induced perturbations of PP2A substrates.

2. KEYWORDS:

PP2A, androgen receptor, prostate cancer, small molecule, xenograft

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

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	Timeline	Site 1	Site 2	Site 3
Specific Aim 1: Determine the effects of DT-061 on prostate tumor growth, and downstream markers of target engagement including AR expression, <i>in vivo</i> .	Months 1-36			Dr. Plymate
Subtask 1: Obtain IACUC approval	1-2			Dr. Plymate
Subtask 2: Implant LuCaP 35, LuCaP 35CR, LuCaP 35 abiraterone resistant, LuCaP 86.2, VCaP, MDVR-VCAP, LNCaP and LNCaP 95 tumors in SCID mice (288 mice). Treat with DT-061.	3-15			Dr. Plymate
Subtask 3: Analyze tumor tissues from mice in subtask 2. Frozen tissue for steroid levels by mass spec, DT-061 levels, AR-FL and AR-SV expression by qRT-PCR and protein. AR canonical and variant transcriptome expression by RNA-seq.	15-36			Dr. Plymate
Milestone(s) Achieved: Proof of concept that DT061 has antitumorigenic effects and	36			Dr. Plymate

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

During the first year of this study IACUC and ACURO approvals were obtained. Our University of Washington 3 year IACUC renewal was also due July 29, 2016. This renewal was completed and submitted and approved July 29th. However, this also prompted an ACURO review of the 3 year renewal. This renewal has been submitted, inquiries are currently being answered, and we are waiting for approval. With permission of ACURO, mice have been purchased and LuCaP86.2 and LuCaP35 xenografts have been implanted. When they reach treatment size (~100-150mm³), gavage will be started with DT-061 and vehicle control.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

During the next reporting period we will expect to have implanted LuCaP86.2, LuCaP35, LNCaP95, and VCaP tumors and have completed treatment of LuCaP86.2 and 35 tumors with analysis.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing To Report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

No changes or problems to report

Actual or anticipated problems or delays and actions or plans to resolve them

Delay due to submission of IACUC 3 year renewal, which required a re-view by ACURO;
delay of approximately 6 months.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

NA

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
Report only the major publication(s) resulting from the work under this award.

Journal publications.

Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Nothing to report

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Example:

Name: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.
Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)

Name: Stephen Plymate, MD
Project Role: PI
Nearest person month worked: 1
Contribution to Project: Oversees all work for grant and prepares manuscripts.

Name: Cynthia Sprenger, PhD
Project Role: Co-Investigator
Nearest person month worked: 3
Contribution to Project: Supervises experimental design, writes animal protocols, assists in data analysis, and assists with manuscripts

Name: Takumo Uo, PhD
Project Role: Research Scientist
Nearest person month worked: 6
Contribution to Project: Assists in data analysis and preparation of manuscripts

Name: Kathryn Epilepsia
Project Role: Research Scientist
Nearest person month worked: 6
Contribution to Project: Oversees all animal work, performs animal procedures and tissue collection
Funding Support: NIH and DOD TIA award

Name: Yan Wang
Project Role: Research Scientist
Nearest person month worked: 6
Contribution to Project: Assists with animal procedures and tissue collection

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: N/A

9. APPENDICES: N/A